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MACINTOSH VERSION IS V6.0c(ENG) AND V6.0Jc(JP),  
AND CURRENT DISCOVER FILE IS DATED 13 JUNE 2005

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FILE 'HOME' ENTERED AT 08:12:27 ON 14 OCT 2005

=> file medline, uspatful, dgene, embase, wpids, biosis  
COST IN U.S. DOLLARS SINCE FILE TOTAL  
ENTRY SESSION  
FULL ESTIMATED COST 0.21 0.21

FILE 'MEDLINE' ENTERED AT 08:12:49 ON 14 OCT 2005

FILE 'USPATFULL' ENTERED AT 08:12:49 ON 14 OCT 2005  
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=> s thermal hysteresis protein  
L1 196 THERMAL HYSTERESIS PROTEIN

=> s (antifreeze or recrystallization inhibition method)  
L2 10526 (ANTIFREEZE OR RECRYSTALLIZATION INHIBITION METHOD)

=> s l2 and l1  
L3 173 L2 AND L1

=> s l3 and (colligative freezing point depression)  
L4 5 L3 AND (COLLIGATIVE FREEZING POINT DEPRESSION)

=> d 14 ti abs ibib tot

L4 ANSWER 1 OF 5 USPATFULL on STN

TI Nucleic acid sequences encoding type III tenebrio **antifreeze**  
proteins and method for assaying activity

AB Thermal hysteresis proteins and their nucleotide sequences derived from  
the Tenebrionoidea Superfamily which lower the freezing point of a  
solution without effecting the melting point. Related methods for  
preparing said proteins and for providing **antifreeze** or  
recrystallization inhibition properties to a subject formulation.

CAS INDEXING IS AVAILABLE FOR THIS PATENT.

ACCESSION NUMBER: 2005:240509 USPATFULL

TITLE: Nucleic acid sequences encoding type III tenebrio  
**antifreeze** proteins and method for assaying  
activity

INVENTOR(S): Horwath, Kathleen L., Endwell, NY, UNITED STATES  
Easton, Christopher M., Ithaca, NY, UNITED STATES

	NUMBER	KIND	DATE
PATENT INFORMATION:	US 2005208509	A1	20050922
APPLICATION INFO.:	US 2004-917030	A1	20040812 (10)
RELATED APPLN. INFO.:	Division of Ser. No. US 2001-876796, filed on 7 Jun 2001, PENDING		

	NUMBER	DATE
PRIORITY INFORMATION:	US 2000-210446P	20000608 (60)
DOCUMENT TYPE:	Utility	
FILE SEGMENT:	APPLICATION	
LEGAL REPRESENTATIVE:	MARK LEVY & ASSOCIATES, PLLC, PRESS BUILDING, SUITE 902, 19 CHENANGO STREET, BINGHAMTON, NY, 13901, US	
NUMBER OF CLAIMS:	27	
EXEMPLARY CLAIM:	1	
NUMBER OF DRAWINGS:	131 Drawing Page(s)	
LINE COUNT:	9928	

CAS INDEXING IS AVAILABLE FOR THIS PATENT.

L4 ANSWER 2 OF 5 USPATFULL on STN  
TI Nucleic acid sequences encoding type III tenebrio **antifreeze**  
proteins and method for assaying activity  
AB Thermal hysteresis proteins and their nucleotide sequences derived from  
the Tenebrionoidea Superfamily which lower the freezing point of a  
solution without effecting the melting point. Related methods for  
preparing said proteins and for providing **antifreeze** or  
recrystallization inhibition properties to a subject formulation.

CAS INDEXING IS AVAILABLE FOR THIS PATENT.

ACCESSION NUMBER: 2005:173249 USPATFULL  
TITLE: Nucleic acid sequences encoding type III tenebrio  
**antifreeze** proteins and method for assaying  
activity  
INVENTOR(S): Horwath, Kathleen L., Endwell, NY, UNITED STATES  
Easton, Christopher M., Ithaca, NY, UNITED STATES

	NUMBER	KIND	DATE
PATENT INFORMATION:	US 2005150000	A1	20050707
APPLICATION INFO.:	US 2004-916986	A1	20040812 (10)
RELATED APPLN. INFO.:	Division of Ser. No. US 2001-876796, filed on 7 Jun 2001, PENDING		

	NUMBER	DATE
PRIORITY INFORMATION:	US 2000-210446P	20000608 (60)
DOCUMENT TYPE:	Utility	
FILE SEGMENT:	APPLICATION	
LEGAL REPRESENTATIVE:	MARK LEVY & ASSOCIATES, PLLC, PRESS BUILDING, SUITE 902, 19 CHENANGO STREET, BINGHAMTON, NY, 13901, US	
NUMBER OF CLAIMS:	3	
EXEMPLARY CLAIM:	1-38	
NUMBER OF DRAWINGS:	131 Drawing Page(s)	
LINE COUNT:	9857	

CAS INDEXING IS AVAILABLE FOR THIS PATENT.

L4 ANSWER 3 OF 5 USPATFULL on STN  
TI Nucleic acid sequences encoding type III tenebrio **antifreeze**  
proteins and method for assaying activity  
AB Thermal hysteresis proteins and their nucleotide sequences derived from  
the Tenebrionoidea Superfamily which lower the freezing point of a  
solution without effecting the melting point. Related methods for  
preparing said proteins and for providing **antifreeze** or  
recrystallization inhibition properties to a subject formulation.

CAS INDEXING IS AVAILABLE FOR THIS PATENT.

ACCESSION NUMBER: 2002:307900 USPATFULL  
TITLE: Nucleic acid sequences encoding type III tenebrio  
**antifreeze** proteins and method for assaying  
activity  
INVENTOR(S): Horwath, Kathleen L., Endwell, NY, UNITED STATES  
Easton, Christopher M., Ithaca, NY, UNITED STATES

	NUMBER	KIND	DATE
PATENT INFORMATION:	US 2002173024	A1	20021121
APPLICATION INFO.:	US 2001-876796	A1	20010607 (9)

	NUMBER	DATE
PRIORITY INFORMATION:	US 2000-210446P	20000608 (60)
DOCUMENT TYPE:	Utility	

FILE SEGMENT: APPLICATION  
LEGAL REPRESENTATIVE: Mark Levy, SALZMAN & LEVY, Ste. 902, 19 Chenango St.,  
Binghamton, NY, 13901  
NUMBER OF CLAIMS: 40  
EXEMPLARY CLAIM: 1  
NUMBER OF DRAWINGS: 131 Drawing Page(s)  
LINE COUNT: 10082  
CAS INDEXING IS AVAILABLE FOR THIS PATENT.

L4 ANSWER 4 OF 5 USPATFULL on STN  
TI Nucleic acid sequences encoding type III tenebrio **antifreeze**  
proteins and method for assaying activity  
AB A **recrystallization inhibition method** for  
determining the presence, relative concentration, and/or activity of  
thermal hysteresis proteins comprising: providing a proteinaceous  
composition in a solvent to form a test solution; flash freezing said  
solution; raising the temperature of the frozen solution to an  
appropriate annealing temperature that allows for a partial melt, while  
limiting heterogeneity in ice grain sizes within said solution;  
maintaining said frozen solution at the annealing temperature for a  
length of time sufficient to allow for recrystallization; monitoring the  
ice crystal grain size changes over time; and determining the presence  
of functional thermal hysteresis proteins in said solution given the  
retention of significantly smaller ice crystal grain sizes relative to  
at least one control solution.

CAS INDEXING IS AVAILABLE FOR THIS PATENT.

ACCESSION NUMBER: 2002:307828 USPATFULL  
TITLE: Nucleic acid sequences encoding type III tenebrio  
**antifreeze** proteins and method for assaying  
activity  
INVENTOR(S): Horwath, Kathleen L., Endwell, NY, UNITED STATES  
Meyers, Kevin L., Trumansburg, NY, UNITED STATES

	NUMBER	KIND	DATE
PATENT INFORMATION:	US 2002172951	A1	20021121
APPLICATION INFO.:	US 2001-876348	A1	20010607 (9)

	NUMBER	DATE
PRIORITY INFORMATION:	US 2000-210446P	20000608 (60)
DOCUMENT TYPE:	Utility	
FILE SEGMENT:	APPLICATION	
LEGAL REPRESENTATIVE:	Mark Levy, SALZMAN & LEVY, Ste. 902, 19 Chenango St., Binghamton, NY, 13901	
NUMBER OF CLAIMS:	34	
EXEMPLARY CLAIM:	1	
NUMBER OF DRAWINGS:	131 Drawing Page(s)	
LINE COUNT:	10121	

CAS INDEXING IS AVAILABLE FOR THIS PATENT.

L4 ANSWER 5 OF 5 WPIDS COPYRIGHT 2005 THE THOMSON CORP on STN  
TI New cDNA polynucleotide encoding a **thermal hysteresis**  
protein which is a Type III anti-freeze protein derived from the  
Tenebrionoidea Superfamily, useful for providing **antifreeze**  
protection to improve the quality of food.  
AN 2002-090137 [12] WPIDS  
AB WO 200194378 A UPAB: 20020221  
NOVELTY - A cDNA polynucleotide (I) comprising a nucleotide sequence for  
encoding a **thermal hysteresis protein** which  
is a Type III anti-freeze protein derived from the Tenebrionoidea  
Superfamily, is new.

DETAILED DESCRIPTION - INDEPENDENT CLAIMS are included for the following:

- (1) a mRNA polynucleotide (II) comprising a nucleotide sequence for encoding thermal hysteresis proteins derived from the Tenebrionoidea Superfamily transcribed from (I);
- (2) a DNA or RNA probe having a sequence complementary or identical to a sequence of contiguous nucleotides for at least a portion of (I);
- (3) a recombinant vector containing (I);
- (4) a **thermal hysteresis protein**, preferably an endogenous Type III anti-freeze proteins, derived from the Tenebrionoidea Superfamily which lowers the freezing point of a solution without effecting the melting point of the solution;
- (5) a consensus sequence with a nucleotide sequence selected from one of the four 481 nucleotide sequences (S1-S4) defined in the specification;
- (6) a consensus sequence with an amino acid sequence selected from the 133 (S5), 134 (S6), another 134 (S7), another 134 (S8) amino acid sequence defined in the specification;
- (7) a consensus sequence with the 133 amino acid sequence (S9) defined in the specification;
- (8) a primer having a nucleotide sequence selected from P1-P3;
- (9) a method (M1) for producing a polypeptide having **antifreeze** properties comprising forming a cloning vector with a Tm 12.86 family member gene encoding an **antifreeze** polypeptide, transferring genes of the cloning vector into DNA of host cell to create a transformed cell, expressing a mRNA sequence and a translated amino acid sequence from the recombinant expression vector, the sequence being isoforms of the Tm 12.86 *T. molitor* **antifreeze** polypeptide;
- (10) a method (M2) for providing **antifreeze** or recrystallization inhibition properties to a subject formulation comprising incorporating at least 0.1 micrograms to 1 mg of an activated polypeptide into 1 ml of a subject formulation to obtain recrystallization inhibition or 1 mg to 25 mg of the activated polypeptide into 1 ml of a subject formulation to thermal hysteresis;
- (11) a Tm 12.86 antibody/antiserum;
- (12) a **recrystallization inhibition** method (M3) for determining the presence, relative concentration, and/or activity of thermal hysteresis proteins comprising providing a proteinaceous composition in a solvent to form a test solution, flash freezing the solution, raising the temperature of the frozen solution to an appropriate annealing temperature that allows for a partial melt, while limiting heterogeneity in ice grain sizes within the solution, maintaining the frozen solution at the annealing temperature for a length of time sufficient to allow for recrystallization, monitoring the ice crystal grain size changes over time, and determining the presence of functional thermal hysteresis proteins in the solution given the retention of significantly smaller ice crystal grain sizes relative to at least one control solution;
- (13) a method for quantitatively assessing the extent of recrystallization occurring in frozen foods, and the impact of solution additives to inhibit or limit recrystallization according to the process defined in M3; and
- (14) a method for quantitatively assessing and comparing the effectiveness of cryoprotective solutions on the extent of recrystallization occurring in cryopreserved cells, tissues, solutions and the like, according to the process defined in M3.

CGGGATCCCTCACCGACGACAG (P1);  
GAGAGGATAACTAATTGAGCTGCC (P2); and  
CGGGATCCCTGACCGAGGCACAA (P3).

USE - The activated anti-freeze protein is incorporated into:

- (a) plant, produce or fish in an amount sufficient to provide **antifreeze** protection;
- (b) a region of a target tissue in an amount sufficient to provide **antifreeze** protein controlled limited tumor cell or target tissue

cryoinjury during cryosurgery;

(c) hypothermic solutions or bathing media to reduce cold damage in order to provide cryogenic or hypothermic preservation of cells and tissues by incorporating the protein into the cells, tissue, or cell membranes in a controlled amount sufficient to provide **antifreeze** protection;

(d) de-icing formulations or used on surfaces to reduce existing ice buildup or abate the formation of ice buildup on surfaces such as a road, aircraft, household products, cosmetic products, machinery and plant surfaces; or

(e) a food product in an amount sufficient to provide **antifreeze** protection to improve the quality of food by abating freezing of solutions, freezer burn, or degradation due to cold storage.

The polynucleotides for the activated protein are used to create transgenic or gene-modified plants, crops, fish, or animals having greater tolerance to cold climatization. The Tm 12.86 antibody/antisera is used as a screening device to identify positive recombinant plaques containing cloned inserts capable in an expression vector system to produce recombinant products recognized by the antibody/antisera. The Tm 12.86 antibody/antisera which is also used as a screening device to screen cDNA libraries in an expression system, including cross-species cDNA libraries to identify homologous sequences in other species.

M3 is used for concurrent multiple sample testing of solutions which includes the 'sandwich' method; and application via a 96 well plate device (all claimed).

Dwg. 0/8

ACCESSION NUMBER: 2002-090137 [12] WPIDS  
DOC. NO. CPI: C2002-027870  
TITLE: New cDNA polynucleotide encoding a **thermal hysteresis protein** which is a Type III anti-freeze protein derived from the Tenebrionoidea Superfamily, useful for providing **antifreeze** protection to improve the quality of food.  
DERWENT CLASS: C06 D16  
INVENTOR(S): HORWATH, K L; MEYERS, K L; EASTON, C M; MYERS, K L  
PATENT ASSIGNEE(S): (EAST-I) EASTON C M; (HORW-I) HORWATH K L; (MYER-I) MYERS K L; (UYNY) UNIV NEW YORK STATE RES FOUND; (MEYE-I) MEYERS K L  
COUNTRY COUNT: 91  
PATENT INFORMATION:

PATENT NO	KIND	DATE	WEEK	LA	PG
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WO 2001094378	A1	20011213 (200212)*	EN	231	
RW:	AT BE CH CY DE DK EA ES FI FR GB GH GM GR IE IT KE LS LU MC MW MZ NL OA PT SD SE SL SZ TR TZ UG ZW				
W:	AE AL AM AT AU AZ BA BB BG BR BY CA CH CN CR CU CZ DE DK DM EE ES FI GB GD GE GH GM HR HU ID IL IN IS JP KE KG KP KR KZ LC LK LR LS LT LU LV MA MD MG MK MN MW MX NO NZ PL PT RO RU SD SE SG SI SK SL TJ TM TR TT TZ UA UG UZ VN YU ZA ZW				
AU 2001075389	A	20011217 (200225)			
US 2002172951	A1	20021121 (200279)			
US 2002173024	A1	20021121 (200279)			
US 2005150000	A1	20050707 (200547)			
US 2005208509	A1	20050922 (200563)			

APPLICATION DETAILS:

PATENT NO	KIND	APPLICATION	DATE
-----			
WO 2001094378	A1	WO 2001-US18532	20010607
AU 2001075389	A	AU 2001-75389	20010607
US 2002172951	A1 Provisional	US 2000-210446P	20000608

US 2002173024	A1 Provisional	US 2001-876348	20010607
		US 2000-210446P	20000608
		US 2001-876796	20010607
US 2005150000	A1 Provisional	US 2000-210446P	20000608
	Div ex	US 2001-876796	20010607
		US 2004-916986	20040812
US 2005208509	A1 Provisional	US 2000-210446P	20000608
	Div ex	US 2001-876796	20010607
		US 2004-917030	20040812

FILING DETAILS:

PATENT NO	KIND	PATENT NO
AU 2001075389	A Based on	WO 2001094378

PRIORITY APPLN. INFO:	US 2000-210446P	20000608; US
	2001-876348	20010607; US
	2001-876796	20010607; US
	2004-916986	20040812; US
	2004-917030	20040812

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FILE 'MEDLINE, USPATFULL, DGENE, EMBASE, WPIDS, BIOSIS' ENTERED AT  
08:12:49 ON 14 OCT 2005

L1 196 S THERMAL HYSTERESIS PROTEIN  
L2 10526 S (ANTIFREEZE OR RECRYSTALLIZATION INHIBITION METHOD)  
L3 173 S L2 AND L1  
L4 5 S L3 AND (COLLIGATIVE FREEZING POINT DEPRESSION)

=> s l3 and (Tm 12.86)  
L5 6 L3 AND (TM 12.86)

=> d 15 ti abs ibib tot

L5 ANSWER 1 OF 6 USPATFULL on STN  
TI Nucleic acid sequences encoding type III tenebrio **antifreeze**  
proteins and method for assaying activity  
AB Thermal hysteresis proteins and their nucleotide sequences derived from  
the Tenebrionoidea Superfamily which lower the freezing point of a  
solution without effecting the melting point. Related methods for  
preparing said proteins and for providing **antifreeze** or  
recrystallization inhibition properties to a subject formulation.

CAS INDEXING IS AVAILABLE FOR THIS PATENT.

ACCESSION NUMBER: 2005:240509 USPATFULL  
TITLE: Nucleic acid sequences encoding type III tenebrio  
**antifreeze** proteins and method for assaying  
activity  
INVENTOR(S): Horwath, Kathleen L., Endwell, NY, UNITED STATES  
Easton, Christopher M., Ithaca, NY, UNITED STATES

PATENT INFORMATION:	NUMBER	KIND	DATE
APPLICATION INFO.:	US 2005208509	A1	20050922
RELATED APPLN. INFO.:	US 2004-917030	A1	20040812 (10)
	Division of Ser. No. US 2001-876796, filed on 7 Jun 2001, PENDING		

	NUMBER	DATE
PRIORITY INFORMATION:	US 2000-210446P	20000608 (60)
DOCUMENT TYPE:	Utility	
FILE SEGMENT:	APPLICATION	
LEGAL REPRESENTATIVE:	MARK LEVY & ASSOCIATES, PLLC, PRESS BUILDING, SUITE 902, 19 CHENANGO STREET, BINGHAMTON, NY, 13901, US	
NUMBER OF CLAIMS:	27	
EXEMPLARY CLAIM:	1	
NUMBER OF DRAWINGS:	131 Drawing Page(s)	
LINE COUNT:	9928	
CAS INDEXING IS AVAILABLE FOR THIS PATENT.		

L5 ANSWER 2 OF 6 USPATFULL on STN  
 TI Nucleic acid sequences encoding type III tenebrio **antifreeze**  
 proteins and method for assaying activity  
 AB Thermal hysteresis proteins and their nucleotide sequences derived from  
 the Tenebrionoidea Superfamily which lower the freezing point of a  
 solution without effecting the melting point. Related methods for  
 preparing said proteins and for providing **antifreeze** or  
 recrystallization inhibition properties to a subject formulation.

CAS INDEXING IS AVAILABLE FOR THIS PATENT.  
 ACCESSION NUMBER: 2005:173249 USPATFULL  
 TITLE: Nucleic acid sequences encoding type III tenebrio  
**antifreeze** proteins and method for assaying  
 activity  
 INVENTOR(S): Horwath, Kathleen L., Endwell, NY, UNITED STATES  
 Easton, Christopher M., Ithaca, NY, UNITED STATES

	NUMBER	KIND	DATE
PATENT INFORMATION:	US 2005150000	A1	20050707
APPLICATION INFO.:	US 2004-916986	A1	20040812 (10)
RELATED APPLN. INFO.:	Division of Ser. No. US 2001-876796, filed on 7 Jun 2001, PENDING		

	NUMBER	DATE
PRIORITY INFORMATION:	US 2000-210446P	20000608 (60)
DOCUMENT TYPE:	Utility	
FILE SEGMENT:	APPLICATION	
LEGAL REPRESENTATIVE:	MARK LEVY & ASSOCIATES, PLLC, PRESS BUILDING, SUITE 902, 19 CHENANGO STREET, BINGHAMTON, NY, 13901, US	
NUMBER OF CLAIMS:	3	
EXEMPLARY CLAIM:	1-38	
NUMBER OF DRAWINGS:	131 Drawing Page(s)	
LINE COUNT:	9857	
CAS INDEXING IS AVAILABLE FOR THIS PATENT.		

L5 ANSWER 3 OF 6 USPATFULL on STN  
 TI Nucleic acid sequences encoding type III tenebrio **antifreeze**  
 proteins and method for assaying activity  
 AB Thermal hysteresis proteins and their nucleotide sequences derived from  
 the Tenebrionoidea Superfamily which lower the freezing point of a  
 solution without effecting the melting point. Related methods for  
 preparing said proteins and for providing **antifreeze** or  
 recrystallization inhibition properties to a subject formulation.

CAS INDEXING IS AVAILABLE FOR THIS PATENT.  
 ACCESSION NUMBER: 2002:307900 USPATFULL  
 TITLE: Nucleic acid sequences encoding type III tenebrio  
**antifreeze** proteins and method for assaying

activity  
INVENTOR(S) : Horwath, Kathleen L., Endwell, NY, UNITED STATES  
Easton, Christopher M., Ithaca, NY, UNITED STATES

	NUMBER	KIND	DATE
PATENT INFORMATION:	US 2002173024	A1	20021121
APPLICATION INFO.:	US 2001-876796	A1	20010607 (9)

	NUMBER	DATE
PRIORITY INFORMATION:	US 2000-210446P	20000608 (60)
DOCUMENT TYPE:	Utility	
FILE SEGMENT:	APPLICATION	
LEGAL REPRESENTATIVE:	Mark Levy, SALZMAN & LEVY, Ste. 902, 19 Chenango St., Binghamton, NY, 13901	
NUMBER OF CLAIMS:	40	
EXEMPLARY CLAIM:	1	
NUMBER OF DRAWINGS:	131 Drawing Page(s)	
LINE COUNT:	10082	
CAS INDEXING IS AVAILABLE FOR THIS PATENT.		

L5 ANSWER 4 OF 6 USPATFULL on STN  
TI Nucleic acid sequences encoding type III tenebrio **antifreeze**  
proteins and method for assaying activity  
AB A **recrystallization inhibition method** for  
determining the presence, relative concentration, and/or activity of  
thermal hysteresis proteins comprising: providing a proteinaceous  
composition in a solvent to form a test solution; flash freezing said  
solution; raising the temperature of the frozen solution to an  
appropriate annealing temperature that allows for a partial melt, while  
limiting heterogeneity in ice grain sizes within said solution;  
maintaining said frozen solution at the annealing temperature for a  
length of time sufficient to allow for recrystallization; monitoring the  
ice crystal grain size changes over time; and determining the presence  
of functional thermal hysteresis proteins in said solution given the  
retention of significantly smaller ice crystal grain sizes relative to  
at least one control solution.

CAS INDEXING IS AVAILABLE FOR THIS PATENT.  
ACCESSION NUMBER: 2002:307828 USPATFULL  
TITLE: Nucleic acid sequences encoding type III tenebrio  
**antifreeze** proteins and method for assaying  
activity  
INVENTOR(S) : Horwath, Kathleen L., Endwell, NY, UNITED STATES  
Meyers, Kevin L., Trumansburg, NY, UNITED STATES

	NUMBER	KIND	DATE
PATENT INFORMATION:	US 2002172951	A1	20021121
APPLICATION INFO.:	US 2001-876348	A1	20010607 (9)

	NUMBER	DATE
PRIORITY INFORMATION:	US 2000-210446P	20000608 (60)
DOCUMENT TYPE:	Utility	
FILE SEGMENT:	APPLICATION	
LEGAL REPRESENTATIVE:	Mark Levy, SALZMAN & LEVY, Ste. 902, 19 Chenango St., Binghamton, NY, 13901	
NUMBER OF CLAIMS:	34	
EXEMPLARY CLAIM:	1	
NUMBER OF DRAWINGS:	131 Drawing Page(s)	
LINE COUNT:	10121	

CAS INDEXING IS AVAILABLE FOR THIS PATENT.

L5 ANSWER 5 OF 6 WPIDS COPYRIGHT 2005 THE THOMSON CORP on STN  
TI New cDNA polynucleotide encoding a **thermal hysteresis**  
**protein** which is a Type III anti-freeze protein derived from the  
Tenebrionoidea Superfamily, useful for providing **antifreeze**  
protection to improve the quality of food.  
AN 2002-090137 [12] WPIDS  
AB WO 200194378 A UPAB: 20020221  
NOVELTY - A cDNA polynucleotide (I) comprising a nucleotide sequence for  
encoding a **thermal hysteresis protein** which  
is a Type III anti-freeze protein derived from the Tenebrionoidea  
Superfamily, is new.

DETAILED DESCRIPTION - INDEPENDENT CLAIMS are included for the  
following:

- (1) a mRNA polynucleotide (II) comprising a nucleotide sequence for  
encoding thermal hysteresis proteins derived from the Tenebrionoidea  
Superfamily transcribed from (I);
- (2) a DNA or RNA probe having a sequence complementary or identical  
to a sequence of contiguous nucleotides for at least a portion of (I);
- (3) a recombinant vector containing (I);
- (4) a **thermal hysteresis protein**,  
preferably an endogenous Type III anti-freeze proteins, derived from the  
Tenebrionoidea Superfamily which lowers the freezing point of a solution  
without effecting the melting point of the solution;
- (5) a consensus sequence with a nucleotide sequence selected from one  
of the four 481 nucleotide sequences (S1-S4) defined in the specification;
- (6) a consensus sequence with an amino acid sequence selected from  
the 133 (S5), 134 (S6), another 134 (S7), another 134 (S8) amino acid  
sequence defined in the specification;
- (7) a consensus sequence with the 133 amino acid sequence (S9)  
defined in the specification;
- (8) a primer having a nucleotide sequence selected from P1-P3;
- (9) a method (M1) for producing a polypeptide having  
**antifreeze** properties comprising forming a cloning vector with a  
**Tm** 12.86 family member gene encoding an  
**antifreeze** polypeptide, transferring genes of the cloning vector  
into DNA of host cell to create a transformed cell, expressing a mRNA  
sequence and a translated amino acid sequence from the recombinant  
expression vector, the sequence being isoforms of the **Tm**  
**12.86** *T. molitor* **antifreeze** polypeptide;
- (10) a method (M2) for providing **antifreeze** or  
recrystallization inhibition properties to a subject formulation  
comprising incorporating at least 0.1 micrograms to 1 mg of an activated  
polypeptide into 1 ml of a subject formulation to obtain recrystallization  
inhibition or 1 mg to 25 mg of the activated polypeptide into 1 ml of a  
subject formulation to thermal hysteresis;
- (11) a **Tm** 12.86 antibody/antisera;
- (12) a **recrystallization inhibition**  
**method** (M3) for determining the presence, relative concentration,  
and/or activity of thermal hysteresis proteins comprising providing a  
proteinaceous composition in a solvent to form a test solution, flash  
freezing the solution, raising the temperature of the frozen solution to  
an appropriate annealing temperature that allows for a partial melt, while  
limiting heterogeneity in ice grain sizes within the solution, maintaining  
the frozen solution at the annealing temperature for a length of time  
sufficient to allow for recrystallization, monitoring the ice crystal  
grain size changes over time, and determining the presence of functional  
thermal hysteresis proteins in the solution given the retention of  
significantly smaller ice crystal grain sizes relative to at least one  
control solution;
- (13) a method for quantitatively assessing the extent of  
recrystallization occurring in frozen foods, and the impact of solution

additives to inhibit or limit recrystallization according to the process defined in M3; and

(14) a method for quantitatively assessing and comparing the effectiveness of cryoprotective solutions on the extent of recrystallization occurring in cryopreserved cells, tissues, solutions and the like, according to the process defined in M3.

CGCGGATCCCTCACCGAACAG (P1);  
GAGAGGATAACTAATTGAGCTGCC (P2); and  
CGCGGATCCCTGACCGAGGCACAA (P3).

USE - The activated anti-freeze protein is incorporated into:

(a) plant, produce or fish in an amount sufficient to provide **antifreeze** protection;

(b) a region of a target tissue in an amount sufficient to provide **antifreeze** protein controlled limited tumor cell or target tissue cryoinjury during cryosurgery;

(c) hypothermic solutions or bathing media to reduce cold damage in order to provide cryogenic or hypothermic preservation of cells and tissues by incorporating the protein into the cells, tissue, or cell membranes in a controlled amount sufficient to provide **antifreeze** protection;

(d) de-icing formulations or used on surfaces to reduce existing ice buildup or abate the formation of ice buildup on surfaces such as a road, aircraft, household products, cosmetic products, machinery and plant surfaces; or

(e) a food product in an amount sufficient to provide **antifreeze** protection to improve the quality of food by abating freezing of solutions, freezer burn, or degradation due to cold storage.

The polynucleotides for the activated protein are used to create transgenic or gene-modified plants, crops, fish, or animals having greater tolerance to cold climatization. The **Tm 12.86**

antibody/antisera is used as a screening device to identify positive recombinant plaques containing cloned inserts capable in an expression vector system to produce recombinant products recognized by the antibody/antisera. The **Tm 12.86**

antibody/antisera which is also used as a screening device to screen cDNA libraries in an expression system, including cross-species cDNA libraries to identify homologous sequences in other species.

M3 is used for concurrent multiple sample testing of solutions which includes the 'sandwich' method; and application via a 96 well plate device (all claimed).

Dwg. 0/8

ACCESSION NUMBER: 2002-090137 [12] WPIDS

DOC. NO. CPI: C2002-027870

TITLE: New cDNA polynucleotide encoding a **thermal hysteresis protein** which is a Type III anti-freeze protein derived from the Tenebrionoidea Superfamily, useful for providing **antifreeze** protection to improve the quality of food.

DERWENT CLASS: C06 D16

INVENTOR(S): HORWATH, K L; MEYERS, K L; EASTON, C M; MYERS, K L

PATENT ASSIGNEE(S): (EAST-I) EASTON C M; (HORW-I) HORWATH K L; (MYER-I) MYERS K L; (UYNY) UNIV NEW YORK STATE RES FOUND; (MEYE-I) MEYERS K L

COUNTRY COUNT: .91

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PATENT NO	KIND DATE	WEEK	LA	PG
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WO 2001094378	A1 20011213 (200212)* EN 231			
RW:	AT BE CH CY DE DK EA ES FI FR GB GH GM GR IE IT KE LS LU MC MW MZ NL OA PT SD SE SL SZ TR TZ UG ZW			
W:	AE AL AM AT AU AZ BA BB BG BR BY CA CH CN CR CU CZ DE DK DM EE ES FI GB GD GE GH GM HR HU ID IL IN IS JP KE KG KP KR KZ LC LK LR LS			

LT	LU	LV	MA	MD	MG	MK	MN	MW	MX	NO	NZ	PL	PT	RO	RU	SD	SE	SG	SI	SK	SL	
TJ	TM	TR	TT	TZ	UA	UG	UZ	VN	YU	ZA	ZW											
AU	2001075389	A	20011217	(200225)																		
US	2002172951	A1	20021121	(200279)																		
US	2002173024	A1	20021121	(200279)																		
US	2005150000	A1	20050707	(200547)																		
US	2005208509	A1	20050922	(200563)																		

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PATENT NO	KIND	APPLICATION	DATE
WO 2001094378	A1	WO 2001-US18532	20010607
AU 2001075389	A	AU 2001-75389	20010607
US 2002172951	A1 Provisional	US 2000-210446P	20000608
		US 2001-876348	20010607
US 2002173024	A1 Provisional	US 2000-210446P	20000608
		US 2001-876796	20010607
US 2005150000	A1 Provisional	US 2000-210446P	20000608
	Div ex	US 2001-876796	20010607
		US 2004-916986	20040812
US 2005208509	A1 Provisional	US 2000-210446P	20000608
	Div ex	US 2001-876796	20010607
		US 2004-917030	20040812

FILING DETAILS:

PATENT NO	KIND	PATENT NO
AU 2001075389	A Based on	WO 2001094378

PRIORITY APPLN. INFO:	US 2000-210446P	20000608; US
	2001-876348	20010607; US
	2001-876796	20010607; US
	2004-916986	20040812; US
	2004-917030	20040812

L5    ANSWER 6 OF 6 BIOSIS COPYRIGHT (c) 2005 The Thomson Corporation on STN  
 TI    Tracking the profile of a specific **antifreeze** protein and its  
       contribution to the thermal hysteresis activity in cold hardy insects.  
 AB    This study summarizes some important new directions in research on  
       **antifreeze** protein biosynthesis and regulation. It describes the  
       recent development and availability of essential biochemical and cellular  
       tools that make possible more direct cellular investigations, and an  
       assessment of the relationship between **thermal**  
       **hysteresis protein** (THP) levels and **antifreeze**  
       activity (both thermal hysteresis and recrystallization inhibition (RI)).  
       These tools include: 1) the isolation of a specific THP of high activity  
       (designated **Tm 12.86**), and an additional  
       endogenous activating factor of this **antifreeze** protein; 2) the  
       ability to track the cellular and secretory patterns of **Tm**  
       **12.86** immunologically; 3) the use of an *in vitro* fat  
       body cell culture system for direct investigation of cellular events. and,  
       4) a means of quantifying RI behavior of purified **Tm 12**  
       .86, and samples of unknown concentrations of THPs, to provide a  
       more sensitive detection method for **antifreeze** activity at  
       scaled down values associated with the *in vitro* system. In combination,  
       these studies indicate that the adaptation mechanisms contributing to the  
       overall **antifreeze** protein response in a cold hardy insect  
       involves a complex interaction between **antifreeze** proteins and  
       endogenous activators of these proteins. With the availability of these  
       key tools, the details of a precise and seasonal regulation of these  
       **antifreeze** protein/activator interactions, which ultimately

generate an efficient cold hardy response, now have the potential to be worked out.

ACCESSION NUMBER: 1996:538806 BIOSIS  
DOCUMENT NUMBER: PREV199699261162

TITLE: Tracking the profile of a specific **antifreeze** protein and its contribution to the thermal hysteresis activity in cold hardy insects.

AUTHOR(S): Horwath, Kathleen L. [Reprint author]; Easton, Christopher M.; Poggioli., George J., Jr.; Myers, Kevin; Schnorr, Ingrid L.

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